

## AN ECHINODERM VITELLARIA WITH A BILATERAL LARVAL SKELETON: EVIDENCE FOR THE EVOLUTION OF OPHIUROID VITELLARIAE FROM OPHIOPLUTEI

GORDON HENDLER

*Smithsonian Oceanographic Sorting Center, Smithsonian Institution, Washington, D. C. 20560*

### ABSTRACT

*Ophionereis annulata* (Le Conte) possesses a barrel-shaped, yolky, non-feeding vitellaria larva with transverse ciliary bands. However, the larva develops vestiges of skeletal structures that are characteristically present in feeding ophiopluteus larvae but absent from vitellariae. Thus, it is evident that the vitellaria of *O. annulata* is a modified ophiopluteus. The presence of a pluteus-like skeleton in a vitellaria larva is suggestive that the evolution of ophiuroid larval types proceeds in a gradual fashion with a larval skeleton remaining after other ophiopluteus structures are lost. Ophiuroid vitellariae have apparently evolved from ophiopluteus larvae. These findings support Mortensen's (1921) proposal that the lecithotrophic vitellaria is a modified pluteus and contradict the hypothesis (Fell, 1945; Williams and Anderson, 1975) that vitellaria larvae are divergent and distinct from the feeding ophiopluteus larvae.

### INTRODUCTION

Johannes Müller and other prominent zoologists of the 19th century discovered that each class of living echinoderms (with the exception of the crinoids) has a feeding (planktotrophic) larva with a distinctive body plan: the holothuroid auricularia, the asteroid bipinnaria, the echinoid echinopluteus, and the ophiuroid ophiopluteus. Müller (1850) also described a yolky, non-feeding (lecithotrophic) larva of ophiuroids. Because of its shape he called it a vermiform (*wurmformige*) larva; this type of larva was later renamed a vitellaria (Fell, 1945).

Certain species in all living echinoderm classes have yolky, non-feeding, larvae that differ strikingly from the better known feeding larvae. The crinoids that have been reared produce yolky, barrel-shaped vitellariae (also called doliolariae) with four or five transverse bands of cilia. Holothuroids have similar vitellariae (doliolariae), either as the definitive larva or as a secondary larval stage. Ophiuroid vitellariae look very much like the barrel-shaped crinoid and holothuroid larvae and generally have four transverse ciliary bands.

Fell (1945) introduced the term "vitellaria" for the larvae which he considered a divergent series possessing yolky, barrel-shaped bodies and transverse bands of cilia. Williams and Anderson (1975) drew a further distinction between lecithotrophic larvae that retain vestiges of feeding larva structures and a separate group of vitellaria larvae which lack any vestiges of feeding larva structures (such as a single ciliary band, mouth, anus, larval arms, and larval skeleton). In their view, reduced larvae such as *Peronella leseuri* echinoplutei or *Amphiura chiajei* ophioplutei are manifestly unlike the vitellaria larvae of the echinoid *Heliocidaris erythrogramma* or the ophiuroid *Ophioderma brevispinum*. Prior to these works, however, Mortensen (1898, 1921) treated ophiuroid vitellaria larvae as a variety of

ophiopluteus. A consideration of the vitellaria of *Ophionereis annulata* (Le Conte) (Fig. 1A), as discussed below, supports Mortensen's contention that the ophiuroid vitellaria is a modified ophiopluteus.

#### MATERIALS AND METHODS

I collected *Ophionereis annulata* at a depth of 12 m near Taboguilla Island in the Bay of Panama (Central America) on 15 May 1975. Specimens were taken to the Galeta Marine Laboratory of the Smithsonian Tropical Research Institute on the Caribbean Coast of Panama. A female ophiuroid, held in the laboratory in a fingerbowl with sea water, spawned spontaneously two days after collection. Its oocytes were fertilized using a dilute suspension of spermatozoa from the dissected testes of a male specimen of *O. annulata*.

Temperature in the laboratory cultures was 24–26°C, approximately that of the water at the collecting site. The process of larval development in these cultures is described from sketches of live specimens, from fresh squash preparations examined under standard and polarized illumination, and from preserved samples.

#### RESULTS

The ova of *Ophionereis annulata* are round, 0.24 mm in diameter, and pale yellow-green to yellow-brown. They were denser than sea water, settling to the bottom of the culture vessel. Within 1.5 h after fertilization the embryos reached the 8-cell stage, and blastulae 0.27 mm in diameter developed by 5 h. Each blastula almost filled the vitelline membrane and did not move within it.

Swimming larvae were found near the bottom of the culture vessels by 10 h after fertilization, and by 12 h some larvae swam near the surface of the water. A 24-h gastrula was about 0.31 mm long, 0.23 mm wide, and somewhat wedge shaped with a blastopore at the center of the broadened posterior end. It swam with the narrow end foremost, rotating clockwise around the long axis of the body.

Several important changes were noted 36 h after fertilization. The blastopore was no longer visible, a hydropore penetrated the mid-dorsal surface of the larva, and internally, a branching hydrocoel encircled the presumptive oral area.

A triradial level skeleton had appeared in each posterior corner of the larval body by 34 h (Fig. 1B). In the 36-h larva, the pair of spicules had grown, and in specimens 38 h old the three branches of each elongate larval skeletal element presumably corresponded to the body, postoral, and posterolateral rods of the pluteus skeleton (Fig. 1C). The more complex branching pattern of the 48-h larval skeleton may be an indication of the formation of homologues of the posterodorsal, anterolateral, and transverse rods of the ophiopluteus skeleton (Fig. 1D).

A pentaradial ophiuroid rudiment (*i.e.* the developing adult body), on the mid-ventral surface of the 38-h larva, below the branched hydrocoel, possessed a concave central oral area and tube foot buds. The entire surface of the 38-h larva including the ophiuroid rudiment was ciliated. Thickened, more densely ciliated ridges were present at the posterior end of the larval body. Apical cilia longer than the body cilia projected from the anterior end of the larva.

By 48 h the hydropore was no longer visible. At that stage the larva was approximately 0.42 mm long and 0.30 mm wide. The larval skeleton was displaced from the posterolateral corners of the larva, possibly through the growth and torsion of the ophiuroid rudiment preceding metamorphosis. Elements of the adult ophiuroid skeleton were conspicuous in squash preparations. They had appeared as tri-

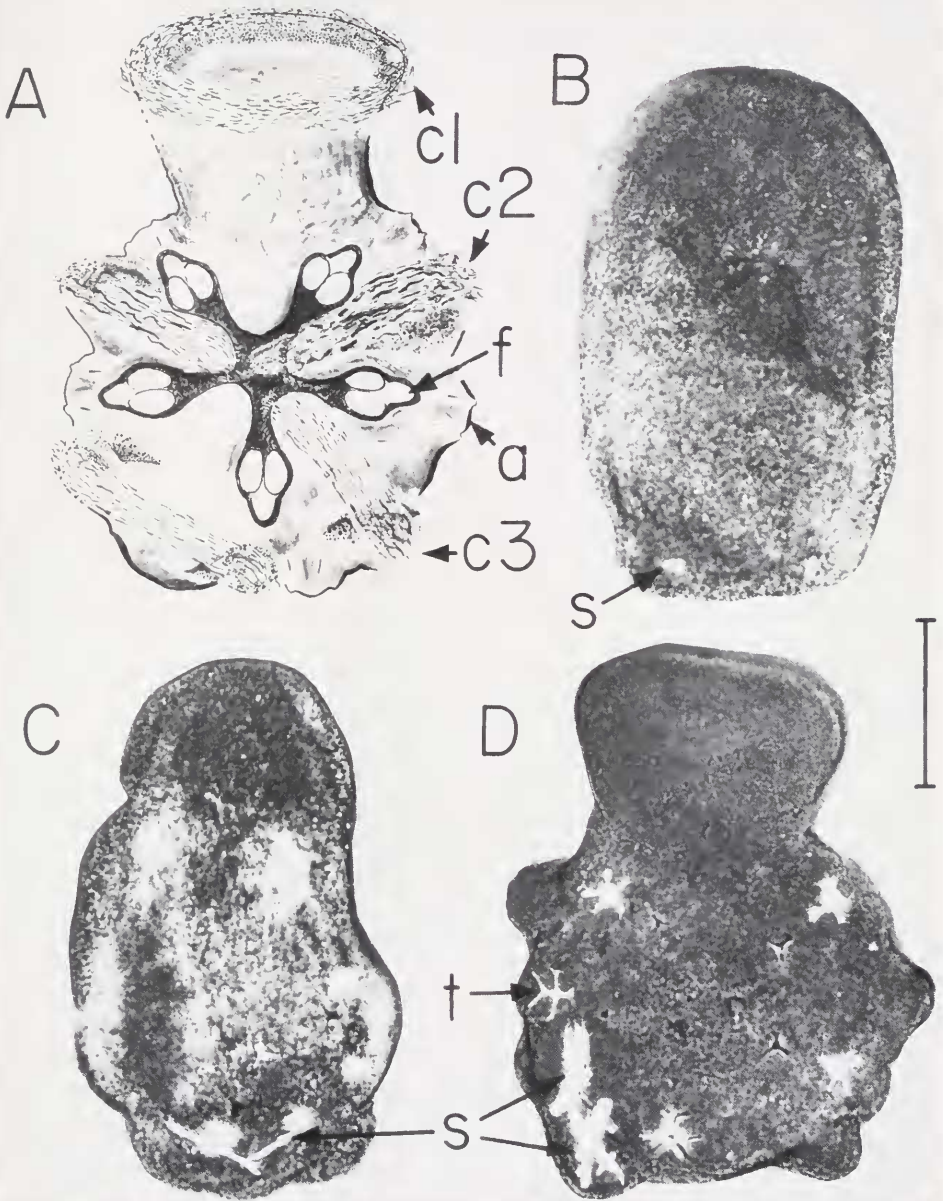


FIGURE 1. (A) 62-hour vitellaria larva of *Ophioneis annulata* drawn from life;  $c_1$ , anterior ciliated band on the funnel-shaped preoral lobe;  $c_2$  and  $c_3$ , posterior ciliated bands on the ophiuroid rudiment; f, triad of the two buccal tube feet and a terminal tube foot lying between the epineural folds; a, arm tip of the ophiuroid rudiment containing a terminal arm plate. B-D are photographs of squash preparations of specimens viewed with polarized illumination that makes skeletal elements appear white and soft tissues of the larva appear dark; s, larval skeleton; t, terminal arm-plate of the definitive ophiuroid skeleton. (B) 34-hour larva with paired posterolateral rudiments of the larval skeleton. The hydrocoel is a scalloped structure at the center of the larva. (C) 38-hour larva with thin, slightly branched larval skeleton and five triradiate terminal arm-plates. Bulging projections at the posterior end of the larva will develop into ciliated bands. (D) 48-hour larva with branched larval skeleton and pentaradiate terminal arm-plates. The preoral lobe and ciliated ridges are seen in silhouette. Length of the scale is 0.10 mm.

radiate spicules by 38 h, and by 42 h they formed multibranched terminal arm plates as well as central, radial, and oral plates of the disc (Fig. 1C).

By 48 h the preoral lobe of the vitellaria formed a funnel shape, giving the larva a distinctive appearance (Fig. 1D). The larva had only three ciliary bands. The absence of a fourth small ciliary band on an elongation of the preoral lobe distinguishes *O. annulata* from other ophiuroid vitellariae, even from the vitellaria of *Ophionereis squamulosa* described by Mortensen (1921).

At 48 h cilia had disappeared from the ophiuroid rudiment. The remainder of the larval body was ciliated, but cilia were concentrated along the transverse ciliary bands. As the ophiuroid rudiment matured, cilia disappeared from most of the larval body, and by 62 h, ciliation was restricted to well-defined bands encircling the larva (Fig. 1A). Ciliary bands on the 72-h larva were opaque yellow-green, but other areas of the larval body, particularly the preoral lobe, had lost their yolky opacity.

The tube feet of 62-h larvae were papilliform, and by 72 h the three tube feet on each arm of the ophiuroid rudiment were capable of independent movement. Tube feet at the tip of the arm protruded from within the terminal arm plate and movements of the paired buccal tube feet set the jaw apparatus in motion.

The 88-h larva was about 0.42 mm long and 0.31 mm wide, approximately the same size as the 72-h larva. At this stage there was scanty ciliation on the ventral surface of the larvae, but lateral ciliation on the ciliary bands was still evident. The ophiuroid rudiment appeared opaque, due at least in part to the growing density of the adult skeleton and the addition of new skeletal structures such as teeth. Larval skeletal elements, however, were completely resorbed by 88 h. They had reached their maximum size by 62 h, and over the next 26 h, the disappearing skeleton remained in one interradial sector of the ophiuroid rudiment.

The newly settled *Ophionereis annulata* moved by propelling the disc with the distal buccal pair of tube feet and the first pair of arm spines. The tips of the tube feet bear papillate extensions, much like the juveniles of *Amphiplus abditus* discussed by Hendler (1977). Within 24 h after settlement, portions of the larval body with yellow-green pigmentation were resorbed and the locomotory activity and agility of the juveniles increased. Within 8 days after settlement the stomach of the juvenile formed a distinct yellow structure within the disc. It is not known whether the pigmentation of the gut was from larval yolk or ingested food.

## DISCUSSION

Fully developed ophioplutei generally have four pairs of larval arms, and the abbreviated pluteus larvae of ophiuroids constitute a continuous reduction series with fewer arms than normal (Fell, 1945; Hendler, 1975). For example, *Amphiura filiformis* has three pairs, and both *Amphiura chajei* and *Ophiothrix oerstedii* have only one pair of larval arms (Mortenson, 1921; Fell, 1945; Fenaux, 1963; Mladenov, 1979). Although the latter two species probably do not feed, they are clearly reduced ophioplutei that retain simplified pluteus arms, skeleton, and ciliation. There are species of brooding ophiuroids (e.g., *Axiognathus squamatus* and *Ophionotus hexactis*) that have embryos with vestigial larval features, and other brooders have embryos lacking pluteus or vitellaria characteristics (e.g., *Ophiomyxa brevirma*) (Mortensen, 1921; Fell, 1941, 1946). The brooded embryos with vestigial ophiopluteus structures have been considered to be modified ophioplutei (Fell, 1946).

Thus, there are reduced ophiuroid larvae which, like the reduced (and secondary) larvae of asteroids, holothuroids, and echinoids, retain certain salient vestiges of the

feeding larva. These larval types would seem to bear no clear relation to the vitellaria larvae since vitellariae lack even vestigial feeding structures and are characterized by their barrel-like shape and multiple transverse ciliary bands that are used solely for locomotion, and not for feeding.

It is therefore surprising that Mortensen (1898) assigned ophiopluteus names to ophiuroid vitellariae, evidently believing that the yolky larvae were modified feeding larvae. Hamann (1901) objected to Mortensen's nomenclature, pointing to a lack of ophiopluteus structures such as larval skeleton in the vitellariae. Later, Mortensen (1921) detected irregular calcareous structures in the vitellariae of the ophiuroid *Ophiolepis cincta*, and he reiterated that the vitellaria was a reduced ophiopluteus. The larval skeleton of *Ophionereis annulata* by its structure and its position in the larva, is more like an ophiopluteus skeleton than are the spicules of *O. cincta* and, therefore, provides better evidence that vitellariae are derived from a feeding larval stage with a bilateral, branched larval skeleton. However, this deduction assumes that vitellaria and ophiopluteus larval skeletons are homologous.

Compelling evidence for homology of the vitellaria skeleton with the ophiopluteus skeleton lies in the fact that both are composed of branching, rod-like forms that develop at the posterolateral corners of the larval body and that are resorbed during metamorphosis. Moreover, the only echinoderm larvae with skeletal rods are vitellariae and plutei, and pluteus larval rods originate in a manner similar to the skeleton of *O. annulata*. It is highly unlikely that the form, location, and ontogenesis of larval skeletons would be duplicated by ophioplutei and vitellariae if the skeletal elements were not homologous.

Assuming that the skeletal structures of vitellariae and ophioplutei are homologous, is there additional evidence that the vitellaria is derived from the ophiopluteus? The necessary logical framework for a solution to this question was devised by Strathmann (1974, 1978) who argued that lecithotrophic larvae are derived from feeding larvae. The most persuasive evidence for the evolution of lecithotrophic larvae from planktotrophic types is furnished by the fact that such a change requires a reduction rather than the repeated acquisition of extremely complex characters such as the single ciliary band feeding mechanism and a complete larval gut (Strathmann, 1974, 1978). Furthermore, some traits of planktotrophic echinoderm larvae are unique to the phylum, whereas lecithotrophic larvae with transverse ciliary bands are simple forms that occur in unrelated taxa, indicating convergence (Jägersten, 1972; Strathmann, 1974).

The ophiopluteus skeleton, like the single ciliary band feeding mechanism, is a complex structure, presumably more readily lost than evolved. Thus, as suggested by the trend of progressive reduction in size and complexity of the larval skeleton shown in ophioplutei with 6, 4, and 2 larval arms, it might be expected that more highly simplified and modified ophioplutei (e.g. vitellariae) could retain some vestige of an ophiopluteus larval skeleton.

As already mentioned, the same reasoning has been applied in treating the relationship between ophioplutei and the specialized embryos of brooding species such as *Axiognathus squamatus* and *Ophionotus hexactis*. The paired skeletal elements in these embryos are regarded as vestiges of an ophiopluteus skeleton. Moreover, the formation of coelomic cavities in the ophiuroid vitellariae also appears to be a simplification of the process of coelomogenesis in the ophioplutei (Grave, 1916). Therefore, I regard the larval skeleton in the vitellaria of *Ophionereis annulata* as a vestigial rather than as a neomorphic (i.e., newly evolved) structure.

The vitellaria of *Ophionereis annulata* provides the best indication that the

ophiuroid vitellaria evolved from an ophiopluteus form. The continuity of the planktotrophic, reduced, and vitellaria larval forms evidenced by the skeleton of the *O. annulata* vitellaria negates the distinction drawn by Fell (1945) and Williams and Anderson (1975) between vitellariae and feeding larvae, and I consider these various ophiuroid larval forms to be homologous.

The presence of a vestigial larval skeleton in *Ophionereis annulata* implies that the loss of feeding larval structures during the evolution of yolky larvae may be a gradual process. Most vitellaria larvae have lost the larval digestive tract, arms, and single ciliary band, as well as the larval skeleton of the ancestral ophiopluteus form. However, the retention of a larval skeleton in *Ophionereis annulata* (and perhaps in *Ophiolepis cincta*) suggests that the larval skeleton may be lost later than other larval structures. In the vitellaria of the related species *Ophionereis squamulosa*, loss of pluteus larval structures is complete. Mortensen (1921) did not mention a larval skeleton in *O. squamulosa* and I have reared *O. squamulosa* (unpub. obs.) and found no trace of the skeleton. Therefore, the larva of *O. squamulosa* is presumably a more advanced form than the larva of *O. annulata*.

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#### LITERATURE CITED

- FELL, H. B. 1941. Probable direct development of some New Zealand ophiuroids. *Trans. Roy. Soc. N. Z.* **71**: 25-26.
- FELL, H. B. 1945. A revision of the current theory of echinoderm embryology. *Trans. Roy. Soc. N. Z.* **75**: 73-101.
- FELL, H. B. 1946. The embryology of the viviparous ophiuroid *Amphipholis squamatus* Delle Chiaje. *Trans. Roy. Soc. N. Z.* **75**: 419-465.
- FENAUX, L. 1963. Note Préliminaire sur le développement larvaire de *Amphiura chiajei* (Forbes). *Vie et Milieu* **14**: 91-96.
- GRAVE, C. 1916. *Ophiura brevispina* II. An embryological contribution and a study of the effect of yolk substance upon development and developmental processes. *J. Morphol.* **27**: 413-451.
- HAMANN, O. 1901. Die Schlangensterne. Pages 751-956 in *Dr. H. G. Bronn's Klassen und Ordnungen des Thier-Reichs*, H. Ludwig, Ed., Vol. 2 (3), C. F. Winter'sche Verlagshandlung, Leipzig.
- HENDLER, G. 1975. Adaptational significance of the patterns of ophiuroid development. *Am. Zool.* **15**: 691-715.
- HENDLER, G. 1977. Development of *Amphiopus abditus* (Verrill) (Echinodermata: Ophiuroidea) I. Larval Biology. *Biol. Bull.* **152**: 51-63.
- JÄGERSTEN, G. 1972. *Evolution of the Metazoan Life Cycle*. Academic Press, New York, 282 pp.
- MLADENOV, P. 1979. Unusual lecithotrophic development of the Caribbean brittle star *Ophiothrix oerstedii*. *Mar. Biol.* **55**: 55-62.
- MORTENSEN, TH. 1898. Die Echinodermenlarven der Plankton-Expedition nebst einer systematischen Revision der bisher bekannten Echinodermenlarven. *Ergebnis. Plankton-Exped. Humboldt-Stift.* **2J**: 1-118.

- MORTENSEN, TH. 1921. *Studies on the Development and Larval Forms of Echinoderms*. G.E.C. Gad, Copenhagen. 261 pp.
- MÜLLER, J. 1850. Ueber die Larven und die Metamorphose der Echinodermen. Dritte Abhandlung. *Abhandl. Königl. Preuss. Akad. Wiss. Berlin*. **1849**: 35-72.
- STRATHMANN, R. R. 1974. Introduction to function and adaptation in echinoderm larvae. *Thalassia Jugoslavica* **10**: 321-339.
- STRATHMANN, R. R. 1978. The evolution and loss of feeding larval stages of marine invertebrates. *Evolution* **32**: 894-906.
- WILLIAMS, D. H. C., AND D. T. ANDERSON. 1975. The reproductive system, embryonic development, and metamorphosis of the sea urchin *Heliocidaris erythrogramma* (Val.) (Echinoidea: Echinometridae). *Aust. J. Zool.* **23**: 371-403.